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14. ABSTRACT

Military traumatic brain injury (TBI) is complex, often involving both diffuse and focal components. The contribution of each of these types of injury to epileptogenic brain activity and ultimately post traumatic epilepsy (PTE) is unclear, as are the mechanisms underlying this transition. Using a large animal model (pig) with adequate white matter pathways and a gyrencephalic brain, we are comparing these injury phenotypes and their potential contribution to PTE. After injury, we chronically implant high density electrodes in the hippocampus, above the cortex near the site of the focal contusion, and ECoG in the contralateral hemisphere. Pigs are monitored via video and electrophysiology up to nine months post injury, and blood biomarkers are being analyzed throughout in order to evaluate them as potential prognostic measures for the development of PTE. A full post-mortem neuropathological examination of axonal and neuronal injury will be performed to have circuitry changes, number and frequency of seizures and inter-ictal events correlated with the neuropathological outcomes to determine the mechanistic underpinnings of PTE.

15. SUBJECT TERMS
Traumatic Brain Injury, Post Traumatic Epilepsy, Contusion, Inertial Injury, Diffuse Brain Injury, Temporal Lobe Epilepsy

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Introduction

Military traumatic brain injury (TBI) is a heterogeneous injury, often involving both focal and diffuse components, and sometimes an accumulation of mild repetitive injuries such as concussion. The high incidence of post-traumatic epilepsy (PTE) is well-established in the military and civilian population. However, the degree to which each of these components of TBI leads to alterations in brain activity that ultimately results in PTE is unknown, as is the mechanism of this transition. In order to address these problems, a pre-clinical model of PTE must accurately reproduce the diffuse as well as the focal components of injury. Since it is known that injury to the axonal connections is a key component of diffuse brain injury, a large animal model (pig) is being utilized to investigate the contributions of each component of traumatic injury to the development of epilepsy. We are therefore developing a large animal model that will determine to what extent each component of the TBI, as well as which combinations, contribute to epileptogenesis. We therefore subject pigs to diffuse and focal injury, as well as combining these injuries. We are also elucidating what abnormal changes in the contused cortex, as well as the circuitry within the hippocampus after injury lead to epileptiform patterns of activity and whether they result in seizures. We are therefore chronically implanting electrodes within the hippocampus and on the surrounding cortex of injured pigs and analyzing changes in the electrophysiology, as well as correlating these findings with post-mortem neuropathology. Finally, we are examining blood biomarkers in this model thought to be indicators of TBI severity to determine if they predict the development of PTE. We are therefore developing a pre-clinical model of PTE that will investigate the mechanisms by which different components of TBI such as focal vs. diffuse injury lead to epileptogenesis. After validation, this new model will serve as a platform for future treatment targets and therapy development.

Keywords

Post Traumatic Epilepsy (PTE)
Traumatic Brain Injury (TBI)
Hippocampus
Epileptogenesis
Electrophysiology
Diffuse brain injury
Focal brain injury
Axonal pathology
Epilepsy monitoring unit
Chronic Implantation
Wireless telemetry
Contusion
Concussion

Accomplishments:

Major Goals

The major goals for this project are captured in the following three Specific Aims in the proposal:

1) Determine the relative contributions of diffuse, repetitive diffuse and focal brain injury to the development of PTE. Epileptogenesis in a large animal model of purely focal and purely diffuse brain injury will be compared with mild repetitive diffuse brain injury and focal injury superimposed on a diffuse injury.
2) Elucidate the circuitry alterations underlying hyperexcitability and epileptogenesis in the above forms of TBI using high density chronic electrophysiology of the hippocampus and cortex, as well as neuropathology.
3) Determine the utility of established blood biomarkers associated with axonal injury in identifying the progressive white matter degeneration leading to epileptogenesis via deafferentation.

Task Summary:

Specific tasks for the first year of these Aims were to validate the contusion injury to complement existing rotational injury, establish chronic electrophysiology of the contusion site and hippocampus as well as video monitoring, injure and implant animals from each injury group, and begin neuropathological characterization of the contusion and rotational injury circuitry changes based on axonal injury. In addition, our goal was to develop the SNTF/GFAP biomarkers in this timeframe. While our first year schedule for the SOW was very aggressive and did not include some needed development steps such as modifying the contusion injury, we have still managed to meet many of the above goals in this timeframe, as well as exceeding some specifications for our recording setup. We have developed and characterized the contusion injury, developed appropriate electrodes for the study and implanted them in pigs for most of the groups proposed (see below), and are currently monitoring them with both EEG and video. In addition, we have characterized the neuropathology of the contusion site, as well as examined historic rotational injuries to begin to assess axonal pathology for the rotation only animals in the temporal lobe circuitry. For the biomarker studies, we have collected bloods from all animals at the times described, and assessed the use of SNTF and another marker (NF-H) as potential PTE biomarkers, but have not yet assessed GFAP (see below). We are in the process of analyzing the electrophysiological data from these animals, as well as transitioning the data analytics to the off-line platform that will be utilized for seizure detection in Dr. Brian Litt's laboratory.

Specific Objectives, Major Activities, Results and Conclusions:

A) Contusion injury validation and neuropathology
B) Grid electrode development and testing
C) Wireless Large Animal Custom Enclosure System (LACES) development and deployment
D) Chronic implantation of Sham and Injured animals, Enrollment
E) Pig Epilepsy Monitoring Unit development and Chronic Monitoring
F) Collection and testing of blood biomarkers for TBI in the pig model
G) Neuropathological examination of axonal injury in rotational injury archival tissue

A) Contusion injury validation and neuropathology:

We had originally planned to perform a negative pressure injury as was performed previously by Smith laboratory. However, after discussion and examination of the variation in the pathology volume in the cortex, and consultation with Dr. Susan Margulies who performs contusion injuries regularly in piglets, it was decided that a cortical impact model would achieve the blood brain barrier (BBB) breakdown and contusion effects with a more precise injury foci. This would then allow for better reproducibility as well as presumed fidelity to cortical contusions. The injury device that is utilized for these injuries was predominantly developed for juvenile pigs. We have adapted the injury device pictured (**Fig.1**) and tested it on two age appropriate Yucatan miniature

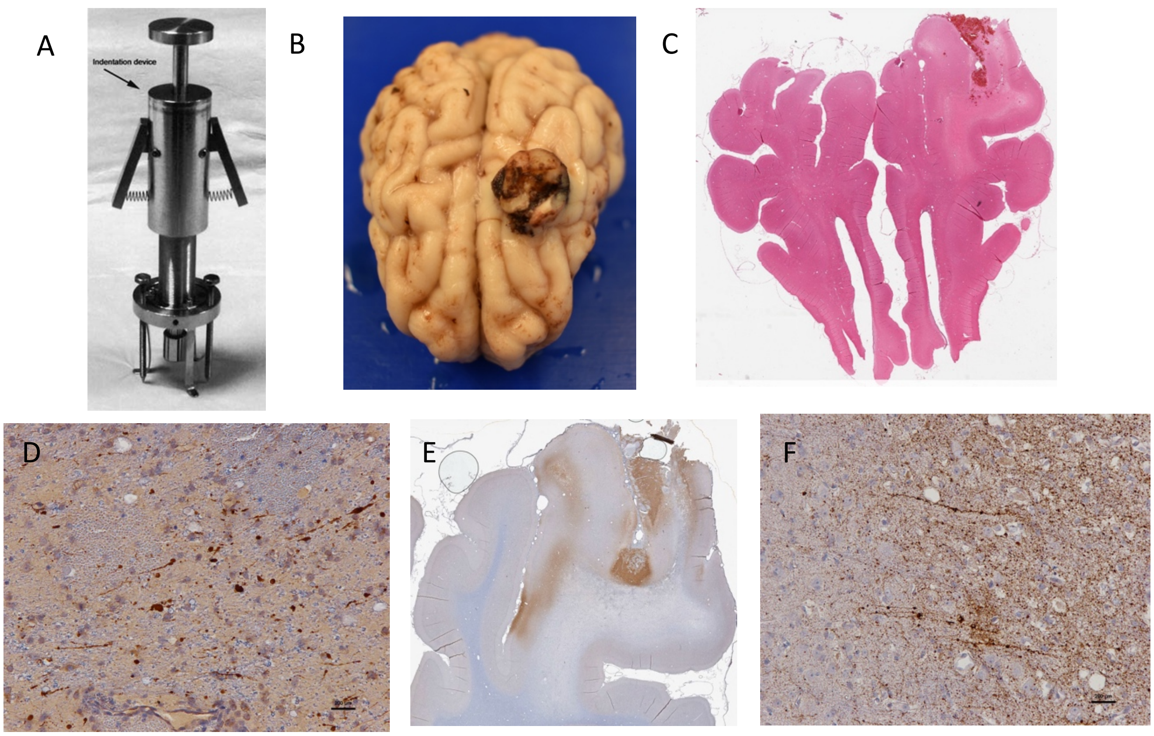


Fig.1 Cortical Contusion Injury. A) Contusion device utilized is well characterized impact device. Animal was survived for 48 hours in order to examine gross and histopathology. B) Gross pathology demonstrated contused region with apparent swelling and partial extrusion through craniotomy. C) H&E staining demonstrates blood present deep in the sulci, loss of staining in adjacent gyri as well. D) APP labeling for damaged axons demonstrates significant axonal injury in the white matter below the injury. E) SNTF labeling appears deep in the injured and adjacent gyri, suggesting a different population of injured axons and dendrites with this labeling. F) Higher magnification of the SNTF labeled population suggests different morphology than APP labeling. Overall the extent and depth of the injury into the white matter is consistent with our injury goals for this project.

swine. The injury appeared to induce little damage at the time of contusion, with only minor surface hemorrhage where the impact occurred. We survived the animals until the 48 hour time point in order to characterize the extent of the pathology, and to examine the brain at the time of implantation in the chronic study animals. The initial assessment at the time of injury was misleading, as the brain had swollen extensively in response to the injury, and extensive effects of the injury to the targeted gyrus and the adjacent gyri were grossly apparent. The neuropathological assessment was performed with H&E, APP, and SNTF as proposed. Red blood cells from the contusion are apparent deep in the gyri, and axonal pathology is present in the depths of the gyri as evidenced by SNTF and APP staining. Interestingly, these appear to be different populations of axons and dendrites that are stained (**Fig.1**). Importantly, localization between the two animals

was comparable, suggesting reproducibility in the model. Blood brain barrier breakdown spread well beyond the area of axonal pathology using fibrinogen staining, suggesting a potential mechanism for epileptogenesis beyond the initial regional damage. Damage to the white matter in both of these gyri, as well as the contusion to the cortical surface, should be sufficient injury to produce an associated epileptogenesis locally that will be captured by the grid electrode. Electrophysiological alterations are present in the adjacent tissue as well (see below).

<u>B) Grid electrode development and testing with depth electrode:</u>

The electrodes first needed to be optimized that are implanted in each of the pigs that is recorded post-injury. These are the hippocampal depth probe, and the cortical micro-ECoG arrays that are being utilized to record activity in the laminar structure of the hippocampus as well as on the cortical surface near the lesion site in the contused animals.

We therefore developed a custom depth electrode with 32 channels (6.1mm in length) to capture changes in the hippocampal circuitry post injury. This electrode has been performing extremely well, giving both units and fields post injury up to 3 months post implantation so far. The grid electrode, which is a less complex design, nevertheless provided the greatest challenge in the project this year.

Our initial goal was to purchase an off the shelf grid from Neuronexus for cost savings and moving the experiments forwards quickly. However, upon receiving these from the company, it was clear that they were really only suitable for use with rodents, with a very thin film and no ability to slide in between the dura and the brain since they had little stiffness. We immediately moved to another company, Ripple Neuro, who has an excellent reputation in the electrophysiology space, and had

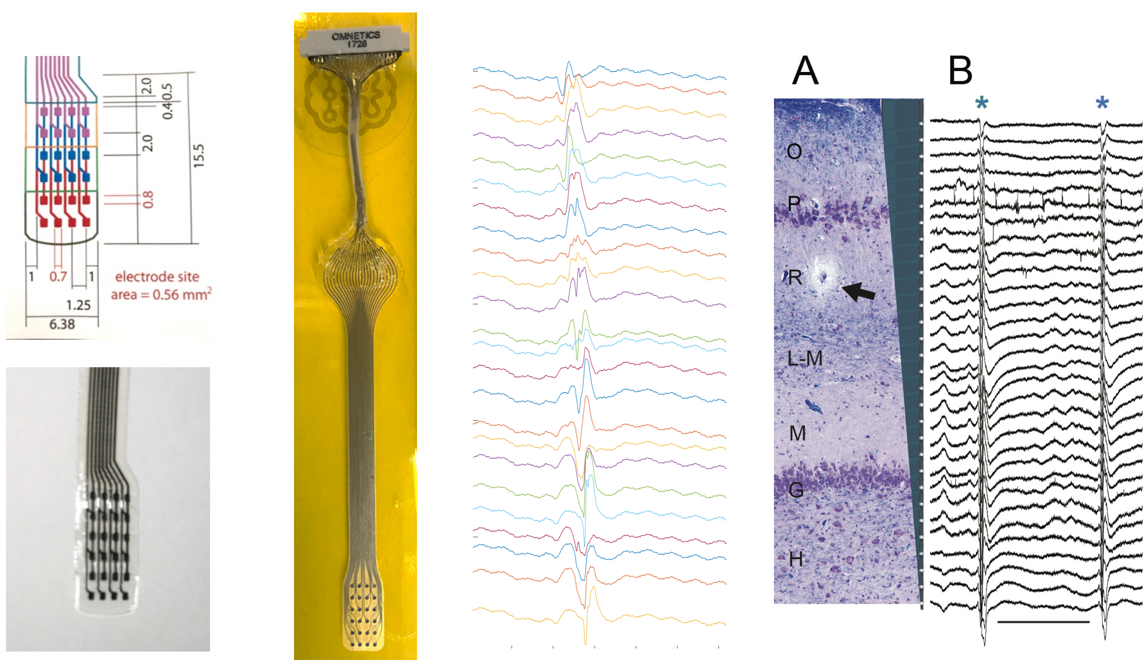


Fig.2. Custom grid electrode design, example grid electrode from Ripple, and the new one from CorTec. 200ms of recordings from the cortical surface of a pig during implantation. Note the differential activity that occurs during the cortical burst, demonstrating the localization of the 300µM electrode contacts. These will be utilized to track the progression of epileptogenic activity from the site of the cortical lesion. On the right is the 6.1mm long depth probe with 32 electrodes that is implanted in the hippocampus.

developed a well tested 3D printing methodology for producing grid ECog electrodes. We designed the electrode above (**Fig.2**) which tested well in initial acute experiments. Unfortunately, our Ripple informed us after our second Quarterly Report that the compound that is utilized to manufacture the grid electrodes had ceased to perform optimally in their testing, and they were delayed by 6-8 weeks in their production of new electrodes. In addition, after chronic testing, a number of channels in each electrode began to fail, potentially due to this issue. We have since recreated the design with a third company, CorTec, who specializes in flexible grids. While there were unexpected costs associated with this, we do not believe there would be any impact to switching manufacturers to the results of the experiments, and consider this issue resolved. We have therefore completed the electrode development for this project, and are continuing with implantations.

C) Wireless Large Animal Custom Enclosure System (LACES) development and deployment

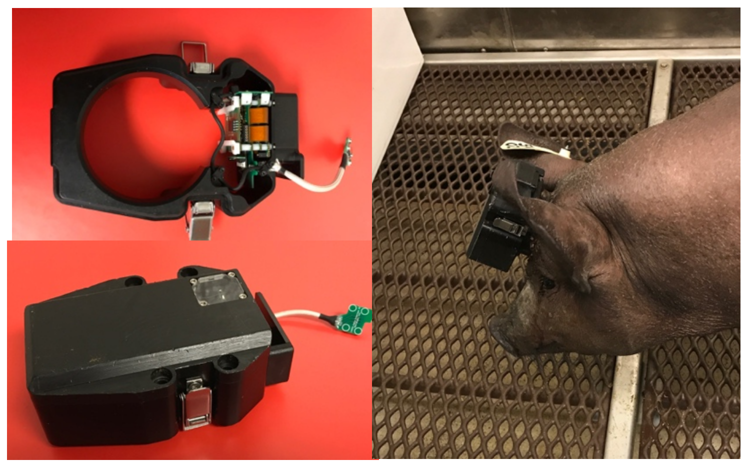


Fig. 3. LACES (Large Animal Custom Enclosure System) for the wireless CUBE2 transmitter. Made of aluminum and delrin, this system allows for the easy change of batteries at the 24hr time point, as well as removal of the SD card (256Gb) for data retrieval. While somewhat bulky due to the 24 hour batteries required, the pig tolerates the implant well even weeks post surgery. The system is designed so that most parts are replaceable over the duration of the 6-9 months of recordings.

It became evident early on with testing of our tethered system and wireless headstages that the wireless system was going to be a longer-term solution and a better solution for overnight recordings. However, the existing system led to the wireless CUBE being exposed to the elements in the cage, easily removed by the pig, and not having large enough batteries for our 12 hour per animal specification in the proposal. We therefore worked with Neuralynx to redesign our wireless headstages and enclosures for implantation so that higher fidelity recordings can be made from the pigs over even longer periods of time (up to 24 hours), termed the LACES enclosure (Large Animal Custom Enclosure System). The initial iteration of the Cube had a battery life that could be adapted up to 12 hours, but had to transmit data continuously to the Cheetah recording system. The new version of the device designed for this project has the ability to record for 23 hours before requiring a change, and importantly can save data directly to a microSD card built in to the unit. In addition, the device has been separated into an analog front end (AFE) which contains the headstage, and the wireless transmitter. Importantly, the AFE headstage is small and inexpensive enough to be permanently mounted on the pigs head, and the transmitter with the SD card and battery can then be swapped out for data downloading and battery changes. This new design has doubled the amount of time that we will be able to record from the pigs during the study, as well as potentially giving us the possibility of recording from all 4 implanted pigs simultaneously should the cost of the transmitter come down (and sufficient funds available through other savings or new funding). A smaller design would have allowed for a less bulky, but would not have provided the same recording time, however we are designing a compromise version to ensure longevity for these experiments (**Fig. 3**).

D) Chronic Implantation of Sham and Injured animals, Study Enrollment and Early Electrophysiology

In order to carry out the experiments in the proposal, the addition of the contusion injury craniotomy and the grid electrode required the redesign of our chronic implantation surgery. We have successfully incorporated the extra surgery for the contusion injury, as well as creating a new EIB board for connecting the electrodes to the LACES enclosures. We performed two preliminary implantations, one each of the depth and grid, for testing of both electrodes and the methodology with the LACES enclosure. As mentioned above, we injured two animals that were sacrificed at 48 hours post injury following the contusion to verify the neuropathological changes in the injury before chronic implantation. An initial animal was also performed with the dura opened using this injury device, but the neuropathology was deemed not severe enough for PTE development, and was therefore altered to be an open dura injury. In addition, the historical animals for rotation were examined for axonal pathology (see below) as part of the study.

We have currently implanted depths with grids in both sham and contusion animals, as well as depth only in the rotational animal as proposed. Our rotational plus contusion pig is currently scheduled for implantation on Nov.2nd with both depths and grids (new from CorTec). As discussed

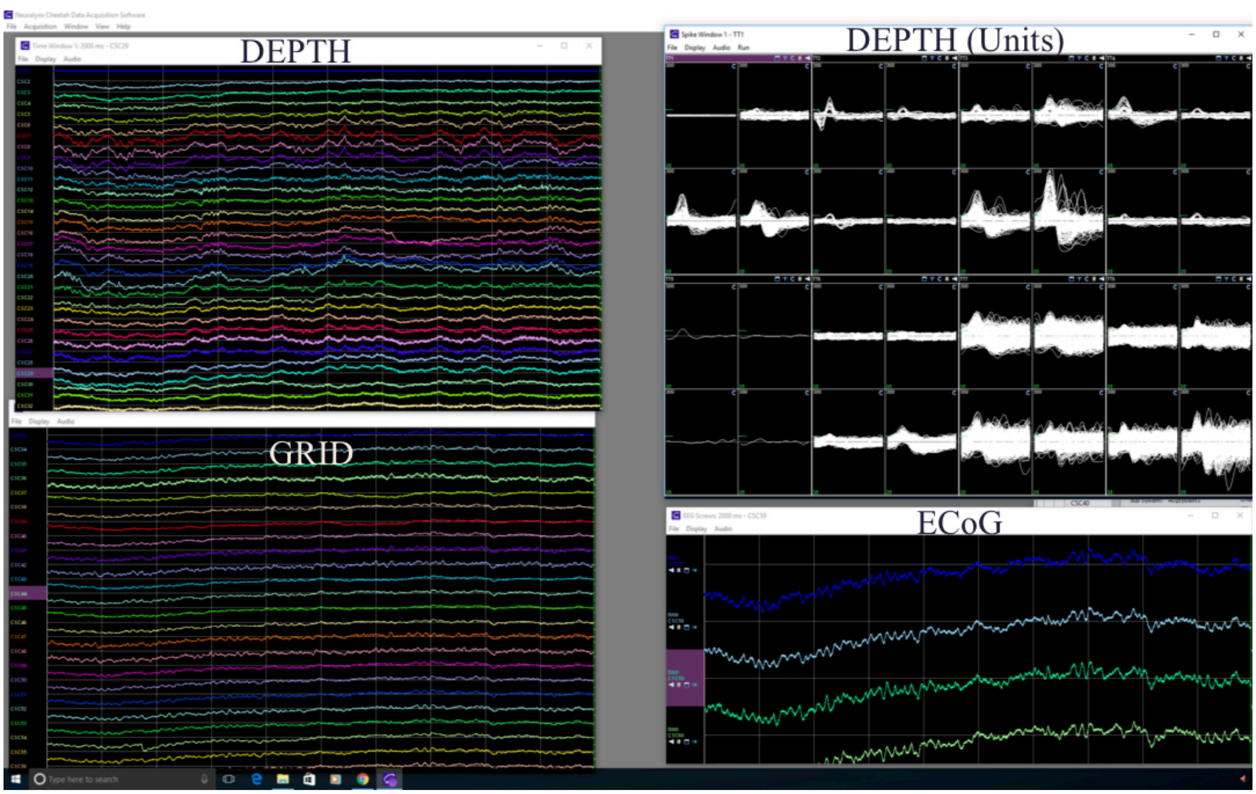
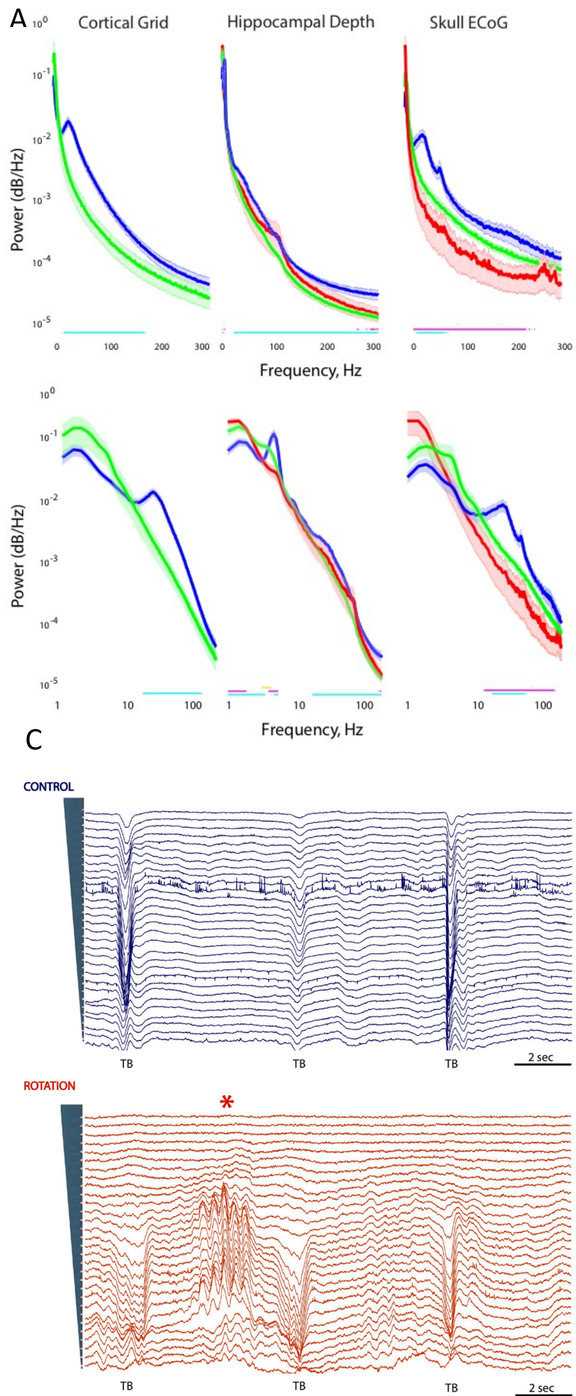


Fig. 4. Awake Wireless Recordings from Chronically Implanted Pig from 3 Types of Electrodes. In a Sham animal, the hippocampal depth is represented in the top left portion, where laminar structure is present on the probe throughout the hippocampus. The grid is underneath, demonstrating changes in the local higher frequency activity between contacts. On the depth there were a number of units present in the days after implantation as well, which may allow for a unique opportunity to examine how individual neurons respond to network level changes following during PTE development. The ECoG panel demonstrates the 3 ECoG electrodes in the contralateral hemisphere, as well as one ipsilateral to the injury site. These will be the equivalent of the traditional monitoring electrodes in PTE experiments. This data was collected with the LACES wireless enclosure and the 64-channel wireless CUBE2 at 30kHz in the animals home cage.

below, the repetitive rotational pig enrollment has been postponed for now as an issue with the bite-plate is resolved. In order to make up for the injury and electrode development time and complete the groups in the current proposal time frame, animals from the last proposed implantation group will be added to each of the next groups, or as animals are removed from the study for epilepsy development, so that the final numbers remain as proposed.

The initial results from the contusion, rotation and sham animals have been compelling. Combined grid and depth recordings from an awake animal are depicted below, as well as the ECoG screws located ispi-and contralaterally to the injury site (**Fig.4**). Note the low noise levels present due to the new LACES enclosure and the wireless system, although the animal is ambulating in its home



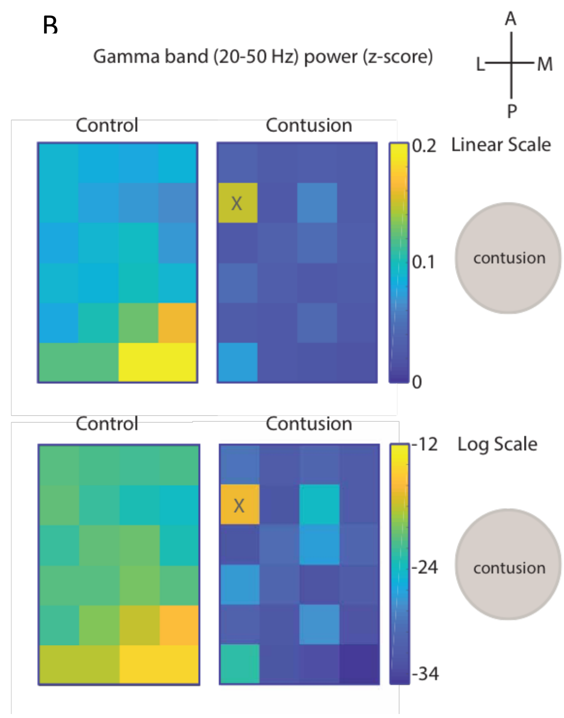


Fig. 5. Changes Post Injury in the Chronically Implanted Animals in Early Recordings. A) Wide-band power levels are significantly attenuated in the injured cortex near the contusion (Green) compared with Sham (Blue). Interestingly, this is also true on the hippocampal depth and some of the power spectrum of the rotational animal (Red) as well. A) bottom - The lower frequency bands also demonstrate the loss of power seen in the higher spectrum in the grid, and there appear to be losses in hippocampal theta (~6hz in pigs) in both injuries, although more robust in the rotational injury. The lower broadband also appears to be attenuated in the ECoG electrodes (epidural). B) The cortical grid comparing sham and control animals demonstrates significant loss of power in the gamma band (20-50Hz) at most channels in the grid, and more prominently on the side closer to the contusion (each electrode on the grid receives one square). Changes in power at higher frequencies in this penumbra of the contusion over time is one of the prominent PTE hypotheses that we will be examining, but the early response is suppression of gamma. C) Hippocampal responses to thalamic bursts are abberant following rotation injury (*).

cage during the recordings. The full spectrum of hippocampal circuitry is present, while the grid depicts the higher frequency activity from the cortex, and EEG like activity on the ECoG electrodes. While we are likely too early in these animals to detect even local seizure activity (6 weeks post in the contusion animal) there are profound changes in the contusion site that may be predictive of later epileptogenesis, as well as changes in the response to cortico-thalamic bursts in the rotational animals (**Fig.5**).

Fig. 6. Pig Epilepsy Monitoring Unit (EMU). Images above demonstrate the video acquisition using IR for 24 hour recordings/monitoring of the pigs (2 shown). Also depicted is the acquisition system for the wireless data, as well as the home cage with the IR illuminators, cameras, and wireless hubs.

<u>E) Pig Epilepsy Monitoring Unit development and Chronic Monitoring</u>

A dedicated room with 5 cages has been provided for the use of this project. We have acquired the appropriate GigE cameras with infrared lighting and filters so that we can monitor the pigs 24/7, and software and a server that is capable of recording from the required number of animals simultaneously with this acquisition equipment. As demonstrated (**Fig.6**), the pigs are readily visible in IR at night and during the day so that appropriate monitoring and seizure/behavioral analysis can be performed without concern for the lighting in the room. We have tested this recording setup over 7 days, and with the above wireless system is consistent enough to be utilized as a daily Epilepsy Monitoring Unit.

<u>F) Collection and testing of candidate blood biomarkers for TBI in the pig model</u>

One of our aims is to test the hypothesis that the brain injury to axonal circuitry is responsible for the development of PTE, and may be identified by a blood test for biomarkers for brain damage. A leading candidate biomarker for the prognosis of PTE is SNTF, a proteolytic fragment (residues 1-1176) of the abundant axonal protein alpha-II-spectrin. SNTF accumulates within damaged axons after human TBI, its blood levels are elevated acutely post-injury and are prognostic for white matter structural abnormalities and persisting brain dysfunction following concussion in humans. However, SNTF has never been evaluated as a blood biomarker for brain injury in the pig, nor tested in the subacute and chronic post-injury periods as a potential prognostic marker for PTE. The SNTF blood test is an electrochemiluminescence-based sandwich immunoassay employing an antibody to the alpha-II-spectrin SH3 domain and a cleavage site-specific antibody specific for the SNTF fragment. We first confirmed that these monoclonals react with SNTF from pig brain, and the latter is highly specific for SNTF exclusively. However, when detection assays for this biomarker were tested using serum from sham, rotation, and contusion injured pigs, detection levels in the blood suggested no difference between sham and injured animals. While

SNTF is present in human blood post-concussion, it is not detectable above baseline levels in brain injured pigs using our current human SNTF capture and detection antibodies. We will continue to assess whether there are other antibodies available that will better detect SNTF in pigs at levels necessary for development of this as a biomarker for SNTF while we pursue other options. We have also tested NF-H, the heavy component of neurofilament as a potential axonal marker for TBI/PTE in this model. This was also not detectable above sham animals in the contused brain with significant axonal neuropathology, suggesting lack of sensitivity of this antibody in this assay. As mentioned above, GFAP is another candidate due to the progression of gliosis in TBI as well as epilepsy. We held off on developing our own capture and detection assay for this target this year, as we learned that the company (Mesoscale) was developing one as well. This assay is now available, and has been ordered for testing to be completed in the next month. In addition, we are investigating the possibility of utilizing NF-light (NF-l) as another potential axonal marker to take the place of the SNTF in this assay, as it has been demonstrated in humans to be a potentially useful TBI axonal injury marker as well.

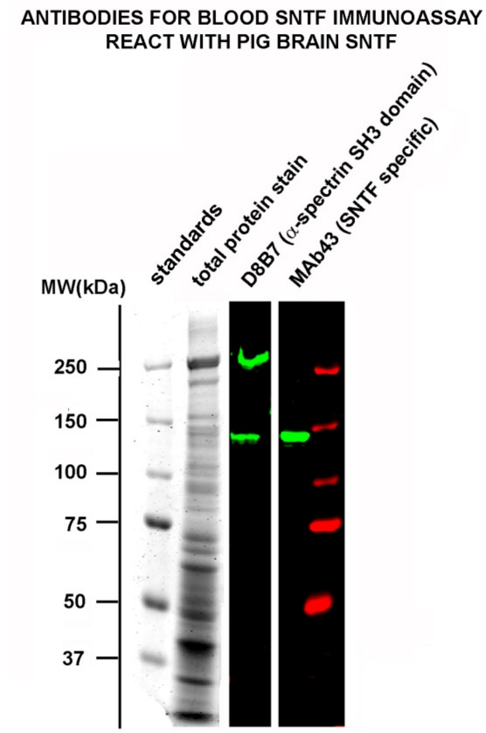


Fig.7. Monoclonals reactive with human SNTF react strongly and specifically to the pig brain protein. SDS-PAGE and Western blot analyses of crude pig brain membranes.
(Left) – Coomassie blue staining for total protein.
(Middle) – Immunoblot using D8B7, a monoclonal reactive with both intact alpha-II-spectrin and amino-terminal fragments containing the SH3 domain.
(Right) – Immunoblot using a cleavage site-specific monoclonal reactive exclusively with SNTF.

G) Neuropathological examination of axonal injury in rotational injury archival tissue

We have quantitatively assessed the axonal pathology in the temporal lobe region of the archival rotational injury animals in order to assess areas of interest for animals being enrolled in the electrophysiology portion of the study. While not unexpected, there was a surprising level of axonal pathology when quantitatively assessed in both the entorhinal region, the fimbria-fornix, and the alveus of the hippocampus, as well as the axonal pathways along the ventricle in this region (**Fig.8**, Table 1).

Table 1: Number of Axonal Profiles at Coronal Hippocampal Level

2016-5	2016-12	2016-13	2017-1	2017-2
Injured	Sham	Injured	Injured	Injured
2202	0	1027	3772	2498

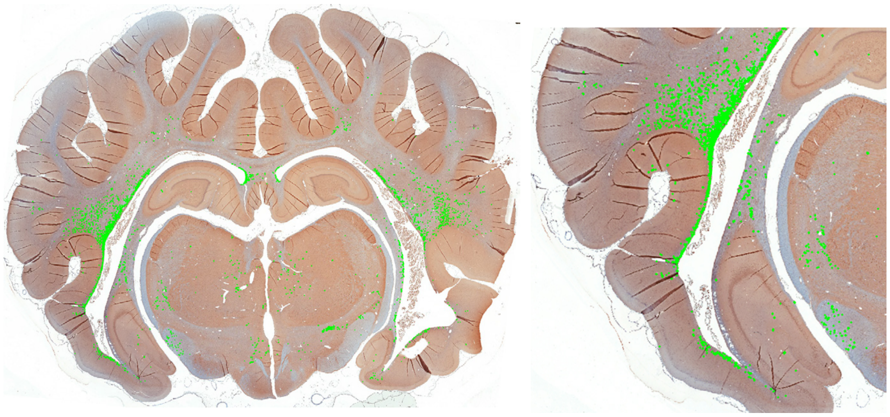


Fig.8. Axonal Pathology from Archive Inertial Injury Animals. APP labeling at 72 hrs post injury allows for quantification of axonal profiles (bulbs and varicosities, marked here in green). Note the diffuse nature of the axonal injury in the white matter, as well as the prominence of axonal pathology in the fimbria-fornix, entorhinal cortex, and pathways leading into the temporal lobe. Assessment of these pathways in our chronically implanted animals may help determine whether deafferentation of the temporal lobe is linked to an increase in TLE post trauma.

<u>Summary of Accomplishments</u>

- Successful design and implementation of depth and grid electrodes for evaluation of pigs following both contusion and rotational injury

- Successful development and deployment of chronically implanted, swappable wireless battery powered system for up to 24 hours of continuous recordings (>12 hrs mentioned in initial proposal)

- Development of an Epilepsy Monitoring Unit for Pigs (PEMU), and successful combination of monitoring of video and these channels post-hoc

- Quantification of axonal pathology in the regions implicated in PTE (deafferentation.)

- Testing of potential biomarkers for PTE in the pig TBI model, although none are sensitive enough currently

- Initial results suggest loss of power in important frequency bands in the contusion and rotational injury in both the grid and depth electrodes

<u>Stated Goals not Met:</u>

While we have optimized the biomarker assay for SNTF and NF-H for pigs, we have not yet assessed the utility of the GFAP marker. This was a conscious decision based on the fact that company that provides immunoassays for the detection device currently utilized (Mesoscale) was in the process of developing an assay for GFAP which would preclude us from having to develop this from scratch. This is now available and on order, and will be tested shortly with our pig samples to assess changes in levels in the injured pig samples. In addition, we have a collaborator with a Quanterix system that has agree to test the pig serum for NF-l, which has recently been examined as another potential axonal based marker for TBI and could therefore also be utilized to correlate with the development of epileptogenesis.

What opportunities for training and professional development has the project provided?

This project was not intended for training and professional development, however has provided excellent opportunities for both the Investigators and the post-doctoral fellows to get more involved in the PTE community, engage with other researchers in the field, and have a significant mentorship component in this area from experts in other areas.

How were the results disseminated to communities of interest?

The current results were disseminated by one talk to a national Functional Neurosurgery conference (H.I Chen, M.D.) and by two poster presentations, one at SFN 2017, and one at AES 2017 (see below.) In addition, this project was presented as part of the CURE Epilepsy 2016 consortium on PTE, where additional funding for the pathological portion of the program was obtained for comparison with human cohorts.

What do you plan to do during the next reporting period to accomplish the goals?

During the next quarter we will finish injury and implantation of the first group of study animals, and continue acquiring data from them including the full Video-EEG configuration that allows for every other day assessment of the animals. In addition, we will test the NF-L and GFAP immunoassay to begin quantification of this biomarkers, as well as collecting serum from the animals pre and post injury at the desired time points. Data structures for storage and analysis of video and EEG will be developed for ease of future analysis, and automated analysis will begin.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

While we have just completed the first year, this project has had an impact on the development of chronic monitoring systems for large animals in PTE, simply by demonstrating that this is possible. The innovative wireless solution to this problem should be translational both up to humans and down to rodents for epilepsy monitoring. This is also the first project to our knowledge that is utilizing both rotational and contusion models and their combination to examine the underlying causes of PTE, and has therefore raised awareness in presenting it that these may be contributions to PTE. To the best of our knowledge, it is the only current program examining PTE in a large animal model as well. The results of the project so far are developmental in nature for the model, but will soon contribute better understanding of the etiology of PTE as we examine the circuitry involved in inertial and contusion models leading to epileptogenesis. In addition, axonal pathology in the temporal lobe in this model suggests an underlying mechanism for the previous reports that diffuse brain injury alone can lead to PTE.

What was the impact on other disciplines?

The chronic implant, laminar electrode technology, and wireless enclosure have significant interest and applications outside of PTE. There are many free roaming large animal experiments that are not being performed currently since this technology is not available. While the goal of this program is the development of a PTE large animal model, we are glad that our contribution to the development of these systems may be translational to other arenas that require freely roaming behavioral, such as social behavior interactions.

What was the impact on technology transfer?

Nothing to report, although the new LACES enclosure and demonstration of a wireless EMU may have an impact on the standard for both animal and human work.

What was the impact on society beyond science and technology?

Nothing to report, although we believe this project will eventually raise public awareness of PTE and potential treatment decision making.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

The initial contusion methodology was discovered to be inconsistent, which required adoption of a more well controlled injury model (Controlled Cortical Impact (**Fig.1**)).

Actual or anticipated problems or delays and actions or plans to resolve them

The adoption of the new contusion model delayed the start of the full group of animals, as did the grid electrode development. We will be adding additional animals to the cohorts to account for this time delay. The initial assessment of our first choice of grid electrode, and the subsequent failure of the supplier of the second, necessitated that another company (CorTec) be contracted to produce the grid electrode. This led to a delay in the initial contusion implantation animals, but should be permanently rectified at this point. We have been testing a new design for our bite-plate that is utilized for the repetitive injuries to distribute strain points on the upper jaw in a more diffuse fashion to reduce orthopedic injuries to the pigs during the second of the two rotations. We have delayed inclusion of the first repetitive injured animal until to accommodate this change in the bite plate, but at this time we do not foresee this changing the enrollment in the study or overall timing.

Changes that had a significant impact on expenditures

The development of the custom electrodes for the grid rather than an "off the shelf" solution led to two "development" charges from both electrode suppliers that were unexpected expenditures. The animal charges for the development of the contusion model were not included in the initial budget.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

PRODUCTS:

Publications, conference papers, and presentations

Platform Presentation: "Contribution of diffuse versus focal injury to the development of epileptiform activity in swine models of TBI." Annual Meeting of the Functional Neurosurgery Society. H. Isaac Chen, M.D.

Abstract: "Acute and chronic in-vivo electrophysiological changes following diffuse and focal traumatic brain injury in a large animal model of post traumatic epileptogenesis."
A. ULYANOVA, P. F. KOCH, C. D. ADAM, M. T. WEBER, D. K. CULLEN, B. LITT, D. H. SMITH, V. E. JOHNSON, H. I. CHEN, J. A. WOLF.
Society for Neuroscience, 2017.

Abstract: "Identifying the contributions of contusion and/or inertial injury to epileptogenesis in a large animal TBI model using a wireless epilepsy monitoring unit."
H. I. CHEN**, A. ULYANOVA,** P. F. KOCH, C. D. ADAM, M. T. WEBER, D. K. CULLEN, B. LITT, D. H. SMITH, V. E. JOHNSON, J. A. WOLF.
American Epilepsy Society

Website(s) or other Internet site(s)

http://www.med.upenn.edu/wolflab/

This site will be the location for the dissemination of the publications and links to the electrophysiology and neuropathological databases that are being generated.

Technologies or techniques

The LACES enclosure was developed with Neuralynx, and will be available through them in addition to the CUBE2 and the system we have developed for chronic implantations.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

We are generating a database of ECoG, grid, and depth electrophysiology with these animals that will be made available to other investigators to analyze. In addition, we are generating a neuropathological archive with these animals that will be the first large animal PTE

neuropathology comparing these two models (inertial and contusion) and we hope will have an impact on the mechanistic understanding of PTE.

In addition, the wireless methodology and the LACES system will have a significant impact in others replicating this work. Our custom grid and depth electrodes will also be made available to the community via Neuronexus (depth) and CorTec (grid).

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: John A. Wolf, Ph.D.
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-6950-2303
Nearest person month worked: 1.8
Contribution to Project: Dr. Wolf is the PI/PD of this project.

Name: Victoria E. Johnson, MBChB, Ph.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2
Contribution to Project: Dr. Johnson is leading the efforts in neuropathology on this project.

Name: H.Isaac Chen, M.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6
Contribution to Project: Dr. Chen is the neurosurgeon on this project, and is contributing his surgical and epilepsy expertise to the design of the implants and to clinical care of the animals.

Name: Brian Litt, M.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0003-2732-6927
Nearest person month worked: 0.6
Contribution to Project: Dr. Litt is heading up the efforts to store and analyze the data from this project using his expertise in seizure detection and cloud storage.

Name: Robert Siman, Ph.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6
Contribution to Project: Dr. Siman is developing the SNTF and GFAP biomarker platforms for the detection of post-injury markers that may be prognostic for PTE.

Name: Alexandra Ulyanova, Ph.D.
Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 10

Contribution to Project: Dr. Ulyanova has decided she will be the post-doc for this project, which we are grateful for as she has experience with PTE and large animal models. She has helped design the Video/EEG paradigms for the chronically implanted animals.

Name: Carlo Cottone, Ph.D.
Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr. Cottone is an expert in the analysis of oscillatory electrophysiology data. He is paid off of an R01, but contributes his effort here as well.

Name: Christopher Adam
Project Role: Research Specialist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Mr. Adam is an expert technician with large animal electrophysiology. He is paid off of another grant, but donates time to this project as well.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Updated Quad Chart Attached.

Diffuse and Focal Brain Injury in a Large Animal Model of PTE: Mechanisms Underlying Epileptogenesis

EP150058
W81XWH-16-1-0675

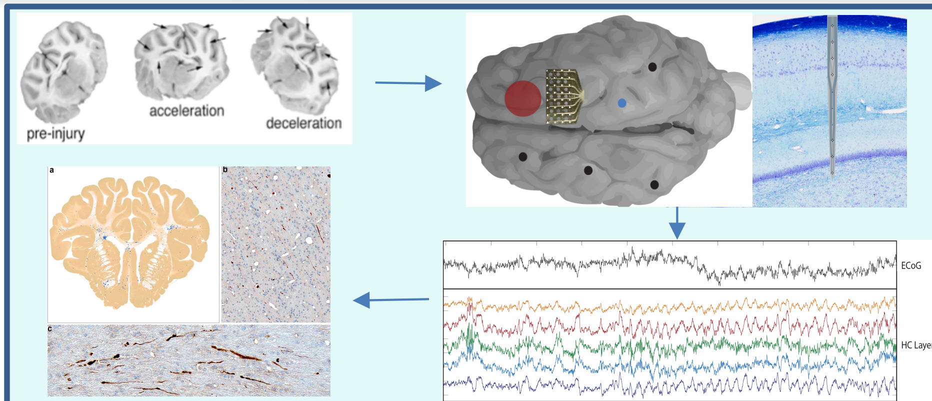
PI: John A. Wolf, Ph.D. **Org:** University of Pennsylvania **Award Amount:** $799,887

Study/Product Aim(s)

• Aim 1: Determine the relative contributions of diffuse, repetitive diffuse and focal brain injury to the development of PTE.
• Aim 2: Elucidate the circuitry alterations underlying hyperexcitability and epileptogenesis in the above forms of TBI using high density chronic electrophysiology of the hippocampus and cortex and neuropathology.
• Aim 3: Determine the utility of established blood biomarkers associated with axonal injury in identifying the progressive white matter degeneration leading to epileptogenesis via deafferentation.

Approach

Epileptogenesis in a large animal model of purely focal and purely diffuse brain injury will be compared with mild repetitive diffuse brain injury and focal injury superimposed on a diffuse injury. We will assess the contribution of each type of injury to the development of PTE, creating the first large animal model of PTE as well as the role of each type of injury. In addition, potential biomarkers for PTE will be assessed.



Pigs will be injured either with an inertial injury, a focal injury (contusion), repetitive inertial, or a combination of inertial and focal injury. These animals will then be implanted with depth and cortical electrodes to monitor epileptogenesis. Biomarkers and neuropathological outcomes will be compared to electrophysiological outcomes.

Accomplishment: We have our IACUC/ACURO protocols approved, and have designed and tested acutely the depth and grid electrodes that will be used for the chronic implantations. In addition, we have determined the type and location of contusion.

Timeline and Cost

Activities CY	16	17	18	19
Approvals/Design of Monitoring				
Implant Group (all injuries)				
Video EEG Monitoring/Analysis				
Assess Biomarkers and Pathology, Compare to EEG				
Estimated Budget ($K)	**$67K**	**$267K**	**$267K**	**$200K**

Updated: 10/29/2017

Goals/Milestones
CY16 Goal – ACURO/IACUC Approval
☑ Electrode Design and Testing
CY17 Goal – Injury and Implantation of Group 1 EEG Monitoring
☑Complete EMU Setup and LACES development
☑All injury types represented and implanted (other than rotation)
☐ Complete Biomarker Development
CY18 Goals – Monitoring and Group 2, 3 Implanted
☐ Monitor with VideoEEG, Neuropath. Correlation to VideoEEG
☐ Comparison of Biomarkers with EEG outcome measures
CY19 Goal – Group 4 implanted, Completion and Analysis
☐ Compare PTE outcomes with Neuropath and Biomarker Predictions
Comments/Challenges/Issues/Concerns
- Grid electrode manufacturer had some delays, workaround in place
- Implanting remaining first group this quarter

Budget Expenditure to Date
Projected Expenditure: $ 266,629
Actual Expenditure: $272,953